Characterization of metabolic health in mouse models of fibrillin-1 perturbation

Tezin A. Walji[a], **Sarah E. Turecamo**[a], **Antea J. DeMarsilis**[a], **Lynn Y. Sakai**[b], **Robert P. Mecham**[a] **and Clarissa S. Craft**[a]

***a* - Department of Cell Biology & Physiology,** *Washington University School of Medicine, St. Louis, MO 63110, USA*
***b* - Department of Biochemistry & Molecular Biology,** *Molecular & Medical Genetics, Oregon Health & Science University, Shriners Hospital for Children, Portland, OR 97201, USA*

Correspondence to Clarissa S. Craft: *at: Campus Box 8228, 660 S. Euclid Ave., St. Louis, MO 63110, USA.*
clarissa.craft@wustl.edu
http://dx.doi.org/10.1016/j.matbio.2016.02.006
Edited by R. Iozzo

Abstract

Mutations in the microfibrillar protein fibrillin-1 or the absence of its binding partner microfibril-associated glycoprotein (MAGP1) lead to increased TGFβ signaling due to an inability to sequester latent or active forms of TGFβ, respectively. Mouse models of excess TGFβ signaling display increased adiposity and predisposition to type-2 diabetes. It is therefore interesting that individuals with Marfan syndrome, a disease in which fibrillin-1 mutation leads to aberrant TGFβ signaling, typically present with extreme fat hypoplasia. The goal of this project was to characterize multiple fibrillin-1 mutant mouse strains to understand how fibrillin-1 contributes to metabolic health. The results of this study demonstrate that fibrillin-1 contributes little to lipid storage and metabolic homeostasis, which is in contrast to the obesity and metabolic changes associated with MAGP1 deficiency. MAGP1 but not fibrillin-1 mutant mice had elevated TGFβ signaling in their adipose tissue, which is consistent with the difference in obesity phenotypes. However, fibrillin-1 mutant strains and MAGP1-deficient mice all exhibit increased bone length and reduced bone mineralization which are characteristic of Marfan syndrome. Our findings suggest that Marfan-associated adipocyte hypoplasia is likely not due to microfibril-associated changes in adipose tissue, and provide evidence that MAGP1 may function independently of fibrillin in some tissues.

Introduction

Microfibrils are extracellular matrix (ECM) structures that contribute to tissue strength and provide instructional signals that affect cellular differentiation and function [4,23,31,32]. These multi-protein filaments appear in early development and are found in almost all tissues [22,25]. The core proteins are the fibrillins, which are encoded by three genes in humans (*FBN*-1, -2, and -3) but only two functional genes in mice (*Fbn*-1, -2) [4,23,32]. Mutations in fibrillin-1 give rise to the autosomal dominant disease Marfan syndrome (MFS), which is associated with musculoskeletal, ocular, pulmonary and cardiovascular anomalies [14,21]. Relevant to this project, individuals with MFS also tend to have reduced fat and muscle mass.

Significant progress toward understanding the molecular pathogenesis of MFS was made with the discovery that the transforming growth factor-beta (TGFβ) signaling pathway was dysregulated in fibrillin-1 mutant mice and that antagonizing this pathway could prevent or reverse pulmonary and cardiovascular phenotypes [10,18,19]. These findings were translated clinically when elevated circulating TGFβ was demonstrated in individuals with MFS [9,17]. Fibrillin-1 mediates TGFβ signaling by stabilizing the latent TGFβ complex in the ECM through direct interactions with the latent TGFβ binding proteins (LTBPs) [12,16,20]. Fibrillin-1 can also regulate TGFβ signaling indirectly through its binding partner MAGP1, which tethers the *active* form of TGFβ to the microfibril [28]. Consequently, loss of MAGP1 also causes activation of the TGFβ signaling pathway [5–7].

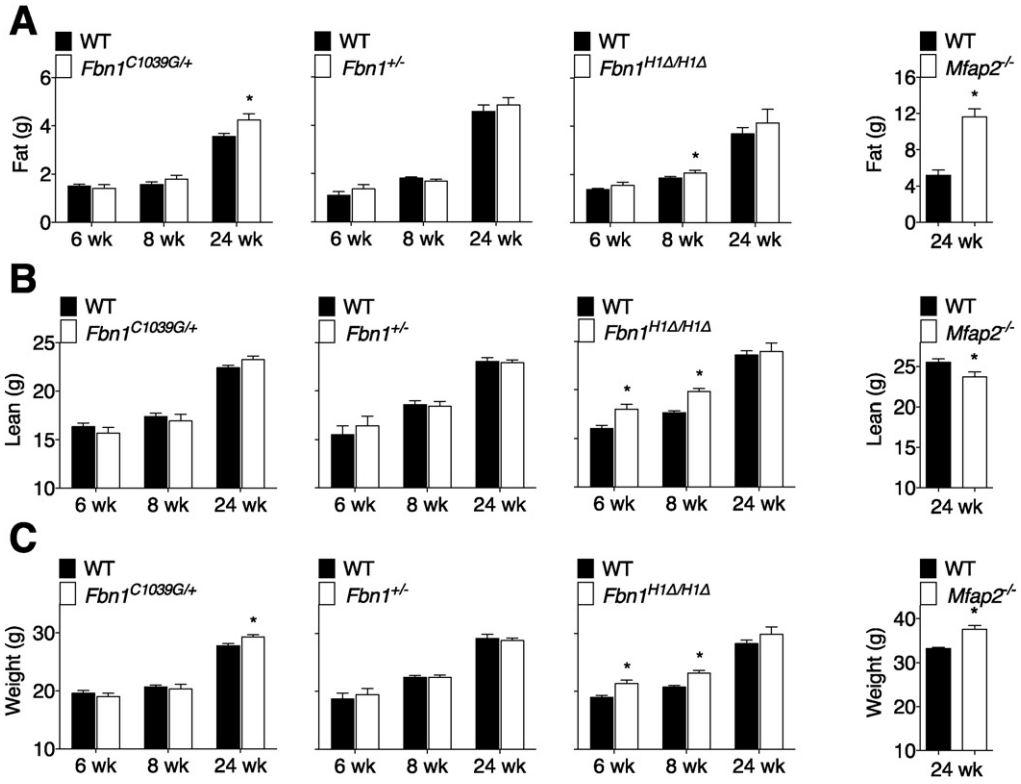

Fig. 1. Fibrillin-1 mutation in mice has little effect on fat and muscle. A–C: Longitudinal study of whole-body fat and lean content was determined by EchoMRI at 6 weeks (A), 8 weeks (B), and 24-weeks of age (C). Mean ± SEM; Mfn: 6 weeks n = 13,8; 8 weeks n = 10,8; 24 weeks n = 10,8. MgN: 6 weeks n = 10,8; 8 weeks n = 13,11; 24 weeks n = 21,20. H1 Δ: 6 weeks n = 8,10; 8 weeks n = 25,18; 24 weeks n = 10,10. MAGP1: 24 weeks n = 6,8. Student *t* test was used for single comparisons (*P ≤ 0.05).

TGFβ has been demonstrated to influence energy expenditure and adipogenesis, and thereby whole body adiposity [15,26,27,29,30]. Serum TGFβ positively correlates with BMI, reducing TGFβ signaling results in leaner mice, and TGFβ blockade can protect mice against diet-induced obesity/diabetes. Increased circulating TGFβ in MFS would therefore be predicted to cause excess adiposity and predisposition to metabolic disease, not reduced adiposity. Thus, extreme leanness associated with MFS confounds our understanding of fibrillin-1's mechanism of action.

This project utilized three mouse models of fibrillin-1 disruption to address fibrillin-1's contribution to adipose tissue homeostasis. The first, fibrillin-1 gene inactivation (*Fbn1*$^{-/-}$), provided a model of fibrillin1-loss of function. Because mice homozygous for the mutation die shortly after birth, we used *Fbn1*$^{+/-}$ animals as a model of fibrillin-1 haploinsufficiency. In the second model, a missense mutation of fibrillin-1 (C1039G) disrupts microfibril assembly and homozygosity of this mutation is also associated with early postnatal death [13]. Therefore, heterozygous mice (*Fbn1*$^{C1039G/+}$) were used as a model of dominant negative effect on microfibril organization and loss of function. The third model utilized mice homozygous for fibrillin-1 with exon 7 deleted (*Fbn1*$^{H1\Delta/H1\Delta}$). Exon 7 encodes the putative site that mediates interaction of fibrillin-1 with LTBPs [20]; these mice assemble microfibrils normally but should not be able to properly sequester the large latent TGFβ complex [3]. The metabolic health of mice associated with these three fibrillin-1 models was compared to mice deficient in MAGP1 (*Mfap2*$^{-/-}$). Loss of this microfibril-associated protein has no consequence on microfibril structure but does result in increased TGFβ signaling in adipose tissue, excess adiposity and metabolic disease [6,28]. Therefore, MAGP1-deficient mice (*Mfap2*$^{-/-}$) represent a reference phenotype for reduced metabolic health that is associated with dysregulated TGFβ signaling in the absence of microfibril structural changes (similar to the *Fbn1*$^{H1\Delta/H1\Delta}$ mouse).

Contrary to expectations based on the human Marfan phenotype, we found that none of the fibrillin-1 mutant mice displayed a lean phenotype; instead, by adulthood these mice tended to be slightly heavier and more insulin resistant than their wild-type littermates. However, these phenotypes are minor relative to the weight gain and metabolic